Molecular epidemiology of *Salmonella* Typhimurium DT104 on Ontario swine farms

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Abstract

This study was conducted to investigate the diversity in antimicrobial resistance (AMR), plasmid profiling, and pulsed field gel electrophoresis (PFGE) of 81 *S.* Typhimurium and S. Typhimurium var. Copenhagen DT 104 strains recovered from pig and environmental fecal samples on swine farms in Ontario. No resistance was observed to amoxicillin and clavulanic acid, apramycin, carbadox, cephalothin, ceftriaxone, ceftiofur, cefoxitin, ciprofloxacin, nalidixic acid, trimethoprim, and tobramycin. However, the isolates exhibited resistance against 4 to 10 antimicrobials with most frequent resistance to sulfonamides (Su), ampicillin (A), streptomycin (S), spectinomycin (Sp), chloramphenicol (C), tetracycline (T), and florfenicol (F). Thirteen distinct AMR patterns and 10 different plasmid profiles were determined. 88% of isolates shared the typical resistance pattern "ACSpSSuT". The isolates were classified into 7 and 18 different genotypes by PFGE-*Spe*I and PFGE-*Bln*I, respectively. However, 23 distinct genotypes were generated by means of PFGE-*Spe*I+*Bln*I. The isolates recovered from pig samples in 18 pens on 10 different farms were discriminated from the isolates recovered from environmental samples from these same pens by PFGE. The highest diversity (discriminatory power) was 0.92 (95% CI: 0.88, 0.93) for PFGE while plasmid profiling had the lowest discriminatory power to differentiate the isolates.

Introduction

Multi-drug resistant *Salmonella* Typhimurium DT104 was first reported from a human case in the UK (Threlfall et al., 1994), and since then it has been isolated from humans and other sources including food-producing animals around the world and has become a worldwide public health concern (Helms et al., 2005). *S.* Typhimurium DT104 first demonstrated a typical penta-resistance pattern to (A), (C), (S), (Su), and (T) but it has more recently displayed additional resistance to other antimicrobials. Multi-drug resistant *S.* Typhimurium DT104 has been also the first or second most common *Salmonella* serovar reported from human and food-producing animals in Canada (Michel et al., 2006; Khakhria et al., 1997; Public Health Agency of Canada, 2004), and it has been found to be associated with increased hospitalization, mortality and consequent economic cost (Martin et al., 2004; Travers and Barza, 2002). During the recent past, *S.* Typhimurium DT 104 has been the most frequent strain isolated in epidemiological studies on swine farms (Farzan et al., 2006; Rajic et al, 2005) and in pork slaughterhouses (Perron et al., 2006). However, since *S.* Typhimurium DT 104 isolates might not be distinguished based on phenotypic characteristics, the source of DT 104 infection in humans has remained unknown. For the control purposes it is very important to understand how the DT 104 isolates are introduced, transmitted, and maintained on farms, as well as to have knowledge of the source-specific attributable fraction for human salmonellosis. Therefore, there is a need for molecular techniques to discriminate *S.* Typhimurium DT 104 strains in order to perform further epidemiological investigation. The objective of this study was to investigate the AMR and molecular characteristics of *S.* Typhimurium DT 104 strains

recovered from apparently healthy pig on swine farms in Ontario. Also the discriminatory power of AMR testing, plasmid profiling, and PFGE for distinguishing DT 104 strains was studied.

Material and methods

In total 81 isolates, including 74 *S.* Typhimurium Copenhagen, 5 *S.* Typhimurium, and one *S.* I:4,12:i:-) isolates recovered from pig fecal samples on 17 swine farms in Ontario were used in this study. The isolates were phage type DT 104 (42 isolates), DT 104a (23 isolates), and DT 104b (15 isolates). Antimicrobial susceptibility of *Salmonella* isolates was tested by the agar dilution method (Poppe et al., 2001). Plasmid finger printing was performed as explained elsewhere (Poppe et al., 2002). PFGE was performed as described previously by the Centers for Disease Control and Prevention (CDC) (2001). Agarose slices containing whole DNA were digested for 18 hr with, *Spe*I and *Bln*I. Results were analyzed with BioNumerics (Applied Maths, Austin, Texas) using the Dice similarity coefficient. Also the similarity coefficient used to create the dendograms using UPGMA with optimization of 1.5% and 2.5% position tolerance. The Simpson's index (Hunter and Gaston, 1988) was used in order to compare diversity among the *S.* Typhimurium DT 104 isolates and the discriminatory power of the methods.

Results

All isolates were susceptible to amoxicillin and clavulanic acid, apramycin, carbadox, cephalothin, ceftriaxone, ceftiofur, cefoxitin, ciprofloxacin, nalidixic acid, trimethoprim, and tobramycin. However, the isolates exhibited resistance against 4 to 10 antimicrobials with most frequent resistance to Su (100%), A (99%), S (99%), Sp (97%), C (96%), T (93%), and F (93%). Thirteen distinct AMR patterns (R-type 1 to 13) were determined (Table). The typical R-type "ACSpSSuT" was common among 88% of isolates. Except for resistance to (T) which was exhibited by 100% of isolates recovered from environmental samples compared to 88% of "pig samples" ($P < 0.05$), and resistance to gentamicin (G) and nitrofurantoin (Nit), which was exhibited only by the strains isolated from pig samples, there was no significant difference in antimicrobial resistance between DT 104 isolated from pig and environmental samples. The resistance to (K) and (N) was significantly correlated to phage type in that 91% of DT104a phage types displayed resistance to these two antimicrobials compared to 19% and 7% of DT104 and DT 104b phage types, respectively ($P < 0.0001$).

Overall, 10 different plasmid profiles (P-type: a to j) were determined (Table). The 62MDa was statistically associated to resistance against (A), (C), (Sp), (S), (Su), and (T) ($P < 0.0001$) and the 2.1 MDa plasmid seemed to be related with resistance against (K) and (N) ($P < 0.0001$). In fact, all isolates susceptible to (K) and (N) lacked the 2.1MDa plasmid while 93% of isolates resistant to these two antimicrobials had this plasmid. The isolates were classified into 7 and 18 different genotypes by PFGE-*Spe*I and PFGE-*Bln*I, respectively. However, 23 distinct genotypes were generated by means of PFGE-*Spe*I+*Bln*I with Dice similarity index between 35% and 100%. In total, the isolates recovered from pig samples in 18 pens on 10 different farms were discriminated from the isolates recovered from environmental samples from these same pens by PFGE. However, these isolates were identical based on phage type, antimicrobial resistance pattern, and plasmid profile. Only isolates recovered from pig and environmental samples from 2 pens on 2 different farms had identical PFGE patterns. The highest diversity (discriminatory power) was 0.92 (95% CI: 0.88, 0.93) for PFGE followed by 0.67 (95% CI: 0.52, 0.77) and 0.56 (95% CI: 0.37, 0.68) for AMR and plasmid profiling, respectively. Except diversity in antimicrobial resistance, there was no significant difference in diversity among the isolates recovered from pig samples compared to those isolated from environmental samples.

Discussion

We found 88% of isolates shared the typical R-type "ACSpSSuT", which has been frequently reported in association to DT 104 isolates from different sources in Canada (Poppe et al, 2002) and other countries (Ridley and Threlfall, 1998; Baggesen et al, 2000; Foley et al., 2006; Gebreyes and Altier, 2002). The variation in AMR between isolates from different sources might represent

some level of true diversity among DT 104 isolates. However, this might be partly due to the between laboratory variation and if this systematic error could be minimized, the *S.* Typhimurium DT 104 isolates might not be differentiated based on the antimicrobial resistance patterns. The 62MDa which was detected in almost 90% of ACSpSSuT-resistant isolates, has been found to carry the *Salmonella* plasmid virulence genes (*spv*), not the antimicrobial resistance genes (Gulig et al., 1987). This indicates a significant correlation between AMR and virulence in *S.* Typhimurium DT 104 isolates. The plasmid profiling and AMR testing had a lower discriminatory power than PFGE which might be due to the instability of the plasmid and lower diversity in extra chromosomal DNA compared to the chromosomal DNA (Fernandez et al., 2003). We used the difference in at least one band to define a genotype. On the other hand, if one defined genotype as the difference in 5-7 bands, which was suggested by Tenover et al (1997), there would then be only one identical clone of DT104 spreading on 17 Ontario swine farms despite the fact that the isolates belonged to three distinct phage types, 15 plasmid patterns, and 15 antimicrobial resistance patterns. We could discriminate DT 104 isolates with similar phage type, AMR, and plasmid profile into different genotypes by PFGE and find a difference in PFGE-genotypes among the DT 104 isolates recovered from pig samples compared to those isolated from environmental samples. These findings might be used to track the source of DT104 on swine farms and find out different gates by which the multi-resistant DT104 is introduced and maintained on swine farms.

Table: AMR, plasmid patterns, and PFGE of 81 DT104 isolates on swine farms in Ontario

Resistance pattern	No. of isolates	Plasmid pattern (MDa)	No. of isolates	PFGE group	No. of isolates
ACFSpSSuT	43	62, 2.1	50	D	12
ACFKNSpSSuT	21	62	21	H	11
ACGKNSpSSu	3	62, 3.0	3	M	10
ACFKNSpSSu	3	4.8, 2.1	1	E	7
ACFNitSpSSuT	2	50, 40, 38	1	S	6
ACFSpSSuT	2	62, 2.8	1	G	4
ACFKNNitSpSSuT	1	62, 36, 2.1	1	K	4
ACFNitSpSSuT	1	62, 4.0, 2.1	1	T	4
AFKNNitSpSSuT	1	65	1	A	2
ACFSpSSu	1	65,1.4	1	C	2
SpSuSxtTm	1			F	2
ACFKNSSuT	1			I	2
ASSuT	1			L	2
				Q	2
				R	2
				B,J,N,O,P,U,V, W	8

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